European Journal of Cell Biology 50, 299–303 (1989)

Secretion of a precursor form of lysosomal α-glucosidase from the brush border of human kidney proximal tubule cells

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Received June 7, 1989
Accepted August 21, 1989

Lysosomal α-glucosidase — kidney brush border — precursor α-glucosidase — kidney proximal tubules — routing of glycoproteins

We have shown previously (R. P. J. Oude Elferink, E. M. Brouwer-Kelder, I. Surya, A. Strijland, M. Kroos, A. J. J. Reuser, J. M. Tager, Eur. J. Biochem. 139, 489–495 (1984)) that human urine contains considerable amounts of a precursor form of lysosomal α-glucosidase (about 50% of the total α-glucosidase activity present). We have now purified α-glucosidase from human kidney. Only about 5 to 10% of the total lysosomal α-glucosidase present in kidney comprises the precursor form of the enzyme. By means of immunocytochemistry using monoclonal antibodies, the precursor of α-glucosidase was detected in the brush border of the proximal tubule cells.

Taking into account the amount of precursor α-glucosidase excreted daily into the urine and the amount present in the kidneys, we conclude that extensive secretion of precursor α-glucosidase occurs from the brush border of the proximal tubules.

Introduction

Lysosomal enzymes are glycoproteins that are synthesized on ribosomes of the rough endoplasmic reticulum. After cotranslational glycosylation of the polypeptides and modification of the oligosaccharide chains in the Golgi apparatus, the lysosomal enzymes have to be segregated from secretory proteins. At least one of the mechanisms for sorting of newly synthesized lysosomal enzymes to the lysosome is the mannose-6-phosphate-dependent pathway (for reviews see [9, 18]). Phosphorylated mannose residues on the lysosomal polypeptides can bind to mannose-6-phosphate-specific receptors in the Golgi apparatus, which is followed by segregation of the polypeptides into specific vesicles.

Under certain conditions and in certain cell types, lysosomal enzymes can also be secreted into the extracellular environment. This applies for instance to fibroblasts cultured in the presence of a weak base [9, 18] or to stimulated macrophages [1].

It has been shown that human urine contains a considerable amount of acid α-glucosidase activity. Schram et al. [16] demonstrated by means of an immunological characterization that this activity is due to lysosomal acid α-glucosidase. It was shown subsequently by Oude Elferink et al. [11] that about 50% of the α-glucosidase activity in urine consists of high molecular weight precursor molecules containing a high amount of mannose-6-phosphate residues. The remaining activity consists of mature α-glucosidase which bears no mannose-6-phosphate on its oligosaccharide chains. In view of the current concepts of the routing of soluble lysosomal enzyme molecules to the lysosome, the question arises of the mechanism(s) responsible for the extensive secretion of precursor α-glucosidase in the urine. Since the α-glucosidase in urine must originate from the kidneys, we have investigated which forms of the enzyme are present in this organ. Using specific monoclonal antibodies, we have furthermore studied the localization of the different biosynthetic forms of α-glucosidase in kidney by immunocytochemical methods.

Materials and methods

Isolation of lysosomal α-glucosidase from human urine and kidney

Isolation of α-glucosidase from human kidney was carried out as described for human urine in [11]. A frozen sample of human kidney was cut into pieces and homogenized in buffer A (50 mM sodium phosphate buffer, 500 mM NaCl, *p*H 6.5) using an Ultra-Turrax homogenizer. The homogenate was centrifuged for 30 min at 50000*g*, and the supernatant was chromatographed on Concanavalin A-Sepharose 4B (Pharmacia, Uppsala/Sweden). The column was washed with buffer A and eluted with buffer A containing 1 M methyl α-D-glucopyranoside (Sigma, St. Louis, MO/USA). The eluate, containing glycoproteins, was concentrated and extensively dialyzed against buffer B (20 mM sodium acetate buffer, 25 mM Na Cl, 1 mM EDTA, *p*H 5.2). The glycoprotein fraction was loaded onto a column (dimensions 120 × 15 cm) of Sephadex G100 (Pharmacia; medium grade). The column was eluted with buffer B at a flow rate of 12 ml/h.

α-Glucosidase activity with p-nitrophenyl-α-glucopyranoside (Koch-Light, Colnbrook/UK) as substrate was measured as described in [11].

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Electrophoresis and immunoblotting

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) and subsequent immunoblotting with a monospecific polyclonal antiserum against α-glucosidase was carried out as described in [13].

Immunocytochemical procedures

Samples of fresh human kidney were obtained from kidneys removed from patients because of the presence of a Gravitz tumor. A part of the unaffected region of the kidney was cut into small pieces and fixed in a mixture of 2% formaldehyde (freshly prepared from paraformaldehyde) and 0.1% glutaraldehyde in a 0.1 M phosphate buffer (*p*H 7.4) for 2 h at room temperature. After fixation, the material was washed three times with 0.1 mM phosphate buffer (*p*H 7.4) and stored in 2% formaldehyde in phosphate buffer at 4 °C until further processing.

Low temperature embedding in Lowicryl K4M was performed according to techniques described elsewhere [2, 15]. Briefly, fixed tissue fragments were dehydrated in a graded ethanol series, during which the temperature was lowered stepwise to −35 °C. Polymerization by UV light was performed for 48 h at −40 °C, followed by 48 h at room temperature.

Two monoclonal antibodies were used in this study; monoclonal antibody 43G8 recognizes all forms of α-glucosidase except the 110 kDa precursor form, whereas antibody 43D1 recognizes all forms of the enzyme [7, 12].

Ultrathin sections were preincubated for 5 min at room temperature on drops of 1% bovine serum albumin dissolved in phosphate buffered saline (*p*H 7.4) (PBS/BSA). Sections were incubated at room temperature successively with one of the monoclonal antibodies dissolved in PBS/BSA for 1 h, with rabbit anti-(mouse IgG) in PBS/BSA for 1 h, and finally with protein A complexed to 10-nm colloidal gold particles [17] in PBS/BSA for 1 h. In control incubations the first antibody was omitted, which resulted in little or no background labeling. After washing with distilled water, the sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate. Sections were examined in a Philips EM 201 or EM 410 electron microscope operating at 80 kV.

Assay for binding of different forms of α-glucosidase to monoclonal antibody 43G8

Protein A-Sepharose beads (about 1 ml packed volume) were incubated either with purified monoclonal antibody 43G8 (see above) or with rabbit anti-(α-glucosidase) for 1.5 h at room temperature. The beads were washed at least four times with PBS. Next, a glycoprotein fraction prepared from human kidney as described above was incubated with the beads in PBS in the presence of 1 mg/ml BSA for 2 h at 4 °C. A parallel incubation was carried out under the same conditions with protein A-Sepharose that had not previously been incubated with antibody. After incubation, the suspension was centrifuged, and activity was measured in the supernatant as described above.

Results

Isolation of α-glucosidases from human kidney

In a previous paper [11] we have shown that α-glucosidase activity from urine can be separated into two fractions upon chromatography on Sephadex G-100. Fraction I contains mainly the precursor form of the enzyme which has an M_r of 110 kDa after SDS-PAGE, whereas fraction II contains only the mature forms of the enzyme (M_r 70 and 76 kDa) [11]. Urinary α-glucosidase most probably originates from the kidneys. For this reason we investigated which molecular forms of the enzyme are present in this organ. An extract of human kidney was prepared and subjected to the same purification procedure as we have described for urine [11]. About 70% of the activity in the kidney extract could be precipitated with a monospecific polyclonal antiserum against the enzyme. The non-precipitable activity probably represents the kidney-specific, non-lysosomal α-glucosidase [4, 5]; this activity did not bind to Concanavalin A and was therefore eliminated during the purification procedure.

A glycoprotein fraction of the kidney homogenate was prepared and chromatographed on Sephadex G-100 (Fig. 1). Most of the enzyme activity eluted in the later fractions, corresponding to fraction II from urine, whereas only a minor peak of activity was recovered in the fractions from kidney corresponding to fraction I from urine. Analysis of both kidney fractions by SDS-PAGE followed by immunoblotting with rabbit anti-(α-glucosidase) revealed similar molecular forms in the two fractions to those found in urine, although the relative amount of precursor was much lower in kidney than in urine.

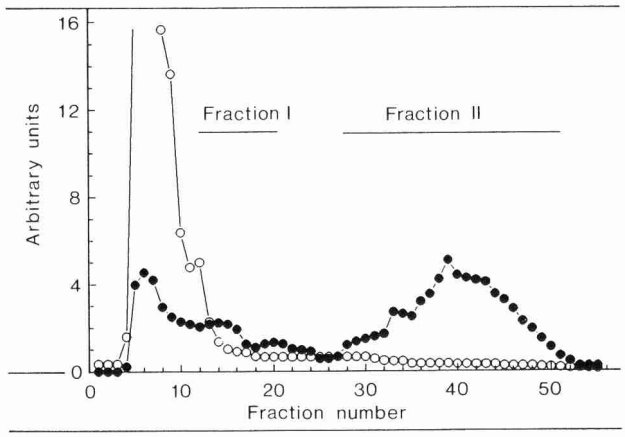

Fig. 1. Sephadex G-100 chromatography of a glycoprotein fraction from human kidney. A glycoprotein fraction from kidney homogenate was prepared as described in Materials and methods. After chromatography on Sephadex G-100, the activity of α-glucosidase was measured with p-nitrophenyl α-glucopyranoside as substrate.

Tab. I. Purification of acid α-glucosidase from human kidney.

Step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Recovery (%)
Homogenate	17.1	—	—	100
Concanavalin A-Sepharose 4B	3.7	54.2	0.068	22
Sephadex G-100 eluate				
fraction I	0.134	0.45	0.30	0.7
fraction II	0.96	0.31	3.10	5.6

For experimental details see Materials and methods. Activity was measured with p-nitrophenyl α-glucoside as substrate.

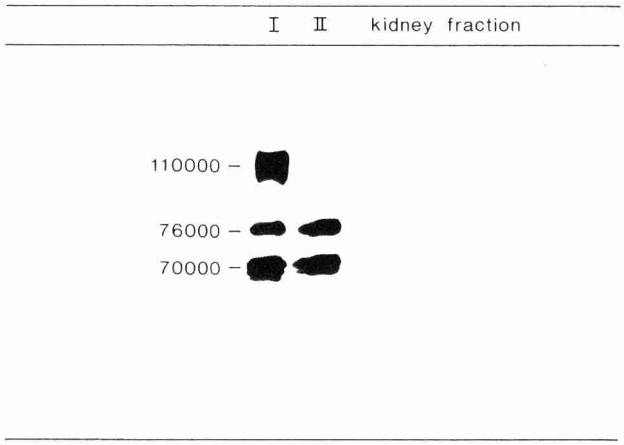

Fig. 2. Western blotting of fraction I and II α-glucosidase from human kidney. Samples from the fractions described in Table I were submitted to SDS-PAGE and immunoblotting with a rabbit antiserum against human placental α-glucosidase. Immunoreaction was visualized with a conjugate of goat anti-(rabbit IgG) with horseradish peroxidase.

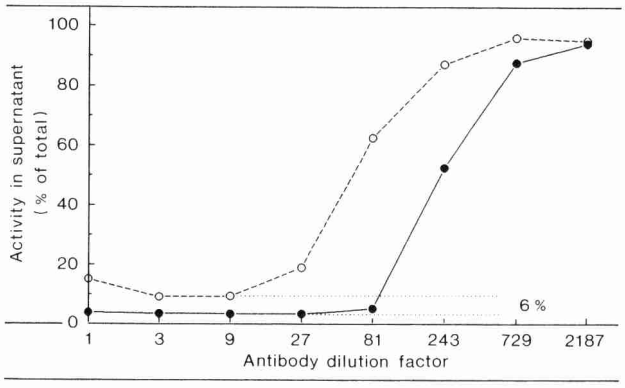

Fig. 3. Titration of α-glucosidase in a kidney glycoprotein fraction with monoclonal antibody 43G8 and polyclonal anti-(α-glucosidase). A glycoprotein fraction from human kidney was incubated with immobilized monoclonal antibody 43G8 (○- - -○) or with immobilized rabbit anti-(α-glucosidase) (●—●) as described in Materials and methods. The suspension was centrifuged, and activity was measured in the supernatant with p-nitrophenyl α-glucoside as substrate.

Table I shows that fraction I from kidney contained about 7-fold lower α-glucosidase activity than fraction II. Furthermore, the immunoblot in Figure 2 shows that a substantial amount of activity in fraction I consisted of mature α-glucosidase.

The activity of α-glucosidase in the kidney glycoprotein fraction (see Materials and methods) was titrated either with monoclonal antibody 43G8, which reacts with all forms of α-glucosidase except the 110 kDa precursor, or with polyclonal anti-(α-glucosidase), which reacts with all forms of the enzyme. The amount of activity precipitated with 43G8 subtracted from the activity precipitated by the polyclonal antiserum is a measure of the amount of precursor α-glucosidase. The results of this experiment (Fig. 3) reveal that about 5 to 10% of the total lysosomal α-glucosidase activity in the kidneys can, indeed, be ascribed to the precursor form.

To localize the precursor of α-glucosidase we performed immunocytochemical labeling experiments with monoclonal antibodies 43G8 and 43D1. With monoclonal antibody 43G8, which does not react with the precursor, only the lysosomes in the proximal tubule epithelial cells were labeled (Fig. 4a). In contrast, when antibody 43D1 was used, which recognizes all forms of the enzyme, additional labeling of the brush border of the proximal tubule epithelial cells was observed (Fig. 4b). These observations lead us to conclude that it is the precursor form of α-glucosidase that is present in the brush border of these cells.

Discussion

Human urine contains a considerable amount of precursor α-glucosidase. This activity cannot be derived from dead kidney cells for the following reasons. The content of lysosomal α-glucosidase of the kidneys is about 0.06 U/g wet weight, which is relatively low compared with the activity found in urine (1.5 U/l). In urine, about 50% of the α-glucosidase activity represents the precursor form (i.e., 0.75 U/l). On the other hand, only 5 to 10% of the kidney α-glucosidase activity represents the precursor form. It can be calculated that two human kidneys (200–300 g wet weight) do not contain more than about 1.1 U of precursor α-glucosidase, which is equivalent to the amount of precursor α-glucosidase in less than 2 l of fresh urine. Since man produces about 1 l of urine daily, it is clearly impossible that the precursor form of α-glucosidase originates from dead cells. Furthermore, the ratio of precursor to mature forms of α-glucosidase in urine is strikingly different from that in kidney, suggesting that different secretion mechanisms exist for the two forms of the enzyme. These findings are in accordance with the suggestion of Kress et al. [10] that two pathways contribute to the appearance of ß-hexosaminidase in urine: a 'lysosomal' pathway leading to excretion of the mature form of the enzyme from the lysosome, and a 'non-lysosomal' pathway leading to secretion of the newly synthesized precursor form, directly from the Golgi apparatus.

Additonal information on this hypothesis was obtained in immunocytochemical experiments. A comparison of the labeling patterns obtained with monoclonal antibodies 43G8 and 43D1 shows that the precursor of α-glucosidase is present in the brush border of the proximal tubule epithelial cells. There are at least two possible explanations for this phenomenon. Firstly, the brush border may be involved in the transport route of lysosomal enzymes from the Golgi apparatus to the lysosomes as originally proposed by Hickman and Neufeld [8]. In this model the precursor present in the brush border would subsequently be endocytosed by these cells. So far, however, no unequivocal evidence has been brought forward for this model. Secondly, the presence of the precursor in the brush border may indicate that extensive secretion of the precursor from

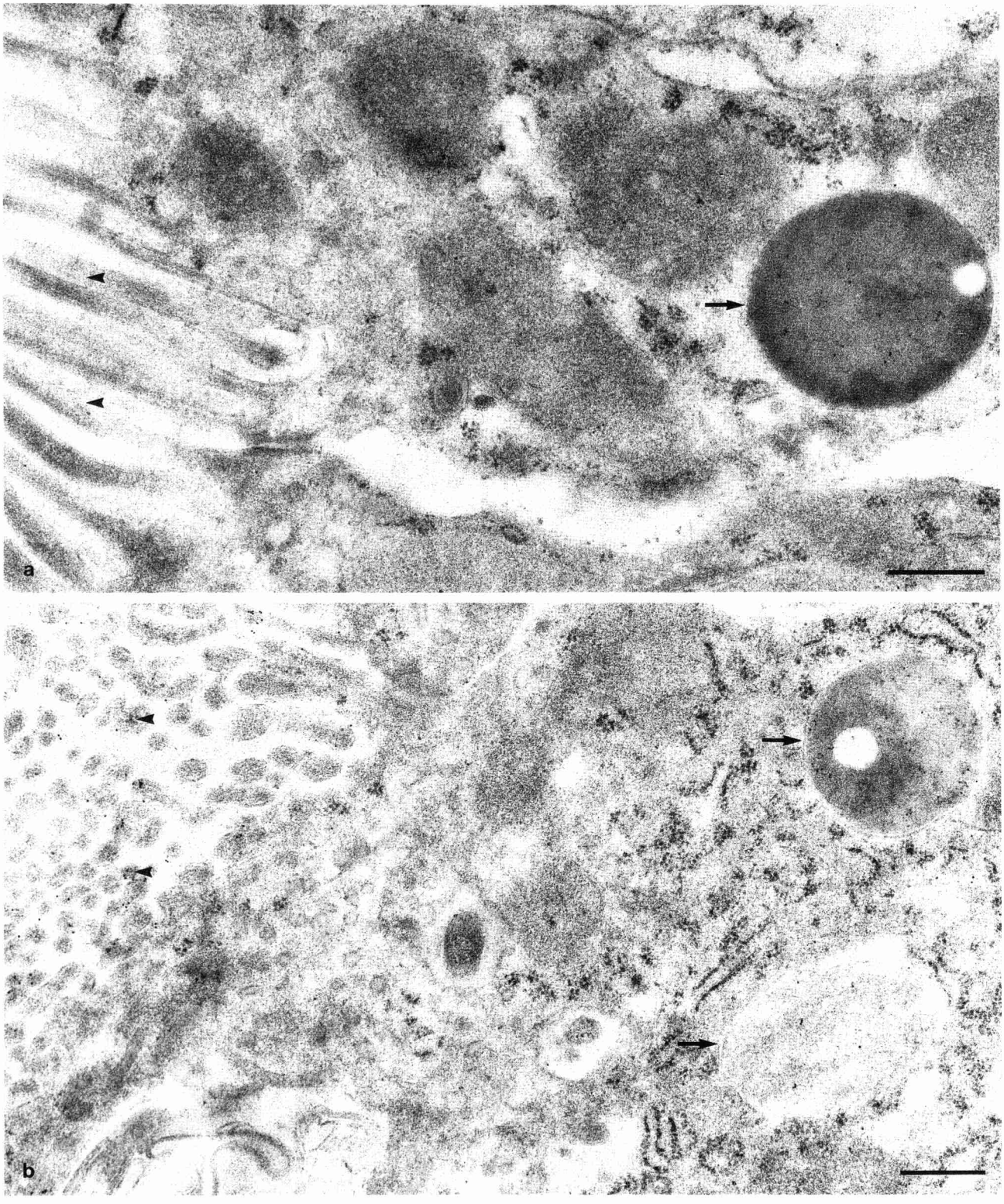

Fig. 4. Localization of the lysosomal enzyme α-glucosidase in proximal tubule epithelial cells. Human kidney was fixed and embedded in Lowicryl K4M as described in Materials and methods. — **a.** Incubated with antibody 43G8, which recognizes all forms except the 110 kDa precursor, results in labeling of the lysosomes only *(arrow)*. The brush border is free of label *(arrowheads)*. — **b.** Incubation with antibody 43D1, which recognizes all forms, results in labeling of the lysosomes *(arrows)* and the brush border *(arrowheads)*, indicating the presence of the 110 kDa precursors of α-glucosidase in the brush border of these cells. — Bars 0.5 μm.

these cells occurs, as was also suggested for intestinal epithelial cells [7].

Since our results have shown that large amounts of the 110 kDa precursor of α-glucosidase are found in urine, we prefer the second explanation. The postulate of a non-lysosomal secretion pathway from the Golgi apparatus directly to the plasma membrane is in accordance with the fact that precursor α-glucosidase is rapidly converted to forms of lower M_r in the lysosome, as has been demonstrated in cultured skin fibroblasts [14], thereby excluding the possibility of excretion from the lysosomes.

The mechanism responsible for extensive secretion of lysosomal enzyme precursors by the kidneys is not clear. In this respect studies carried out by the group of Sly [3, 6] may be of special interest. They have measured the lysosomal enzyme-binding activity (i.e., receptor activity) in different rat organs. Membranes from every organ examined contained different amounts of mannose-6-phosphate-inhibitable enzyme-binding activity. Furthermore, there was a striking correlation between the enzyme-binding activity and the amount of lysosomal enzymes found in the homogenates of these different tissues. On the one hand, this could be explained if one assumes that the expression of receptor(s) and of the lysosomal enzyme are subject to the same regulatory mechanism. On the other hand, if this is not the case, the amount of (soluble) lysosomal enzymes could be regulated by the amount of receptor in a certain cell type; if in this cell type a surplus of lysosomal enzymes is synthesized, as could be the case in proximal tubule cells, this surplus would be secreted because receptors are fully occupied with lysosomal enzymes.

Acknowledgements. The authors are very grateful to Hans Aerts for stimulating discussions and to Wendy van Noppen and Els Vlugt for their help in the preparation of this manuscript.

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